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Biodiversity of free-living flagellates in Kuwait's intertidal sediments

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Abstract

Taxonomic data of free-living benthic flagellates in Kuwait's intertidal sediments are summarized. A full list of the species composition is presented, including distribution on different sediment types, species occurrence and light micrographs for each taxon identified. A total of 67 flagellate species were identified, representing six classes. Most of them are reported from Kuwait for the first time. The most abundant and diverse species were sand-dwelling dinoflagellates (43 taxa).

Keywords

Benthic flagellates, intertidal sediments, Kuwait

Introduction

The marine coast of Kuwait, which extends for about 170 km, is composed of a variety of coastal habitats (Al-Yamani et al. 2004). Kuwait's coast may conveniently be divided into two primary regions, which reflect the position of Kuwait's offshore energy zones. In the north, suspended material from the Shatt al-Arab delta has settled to form extensive intertidal mud and sand flats within the rather protected low-energy zone of Kuwait Bay. Intertidal sediments grade from mud in the north and west of the Bay, where limited salt marshes may occur, to medium or fine sand beaches at the entrance of the Bay. On the more exposed open coast south of Kuwait Bay in high-energy areas, medium and coarse sand beaches extend down to the Saudi Arabian border and beyond. Within these two broad categories, however, there are several other shoreline types, which can grade into one another. Thus, all stages of mud, sandy mud, muddy sand, sand, sandy-rocky flats, or rocky shore can be found along the coastline of Kuwait, often in close proximity of each other (Jones 1986a, b).

This unique and very productive component of Kuwait's marine ecosystem is poorly studied in terms of its microbenthic community. Previous investigations of marine protists in some parts of Kuwait's coastline focused on their general productivity and/or diversity especially for the diatom/cyanobacteria component of the microalgal community (e.g. Hendey 1970, Clayton 1986, Jones 1986b, Hoffman 1996, Al-Yamani et al. 2004, Al-Zaidan et al. 2006), whereas information on taxonomy and ecology of benthic flagellates in Kuwait's coastal zone is totally lacking. Many of the benthic flagellates are abundant in intertidal substrata and their contributions to benthic and shallow marine ecosystems may be significant. Several species from the benthic flagellated community are known to be potentially toxic; therefore, the study of their biology and potential toxic reactions in shallow waters is important.

The aim of this study was to document the biodiversity of benthic flagellates along the Kuwaiti coastline. Additionally, their abundance and distribution on different sediment types are reported.

Methods

We selected 14 localities along Kuwait's coast (Fig. 1) for our investigation. Each study site stretched for about 100 m and consisted of one or more of the following substrates: mud, sandy mud, muddy sand, or sand. In total, we collected 127 sediment samples from different intertidal heights during 2005 to 2007. Using plastic tube corers, we sampled the top 3 to 4 cm of sandy sediment, or 3 to 4 mm of muddy sediment. These samples were transported to the laboratory where flagellates were separated from the sediment using Uhlig's (1964) frozen seawater method and a 110 µm mesh filter. Flagellates were collected in a Petri dish beneath the filter and examined alive with a Leica DMIL inverted microscope at ×35 to ×200 magnification. For detailed observations, flagellates were isolated by micropipette and examined with a Leica DMLM microscope or with a Carl Zeiss Axiovert 200M microscope using transmitted-light with a bright field and phase contrast at ×400 to ×1000 magnification. Flagellate plate patterns were made using Calcofluor White M2R (Fritz and Triemer 1985). The cells were examined on an epifluorescent (violate excitation ca 430 nm, blue emission ca 490 nm) Axiovert 200M microscope. We examined both the dorsal and ventral sides of each flagellate, and obtained micrographs using either Axiovert 200M microscope equipped with an AxioCam HRc digital camera or a Leica DMLM microscope with a Leica DFC 320 digital camera. Cell size was measured by light microscopy using a calibrated ocular micrometer, and some morphometric measurements were obtained from micrographs using Carl Zeiss Axio Visiton 3.0

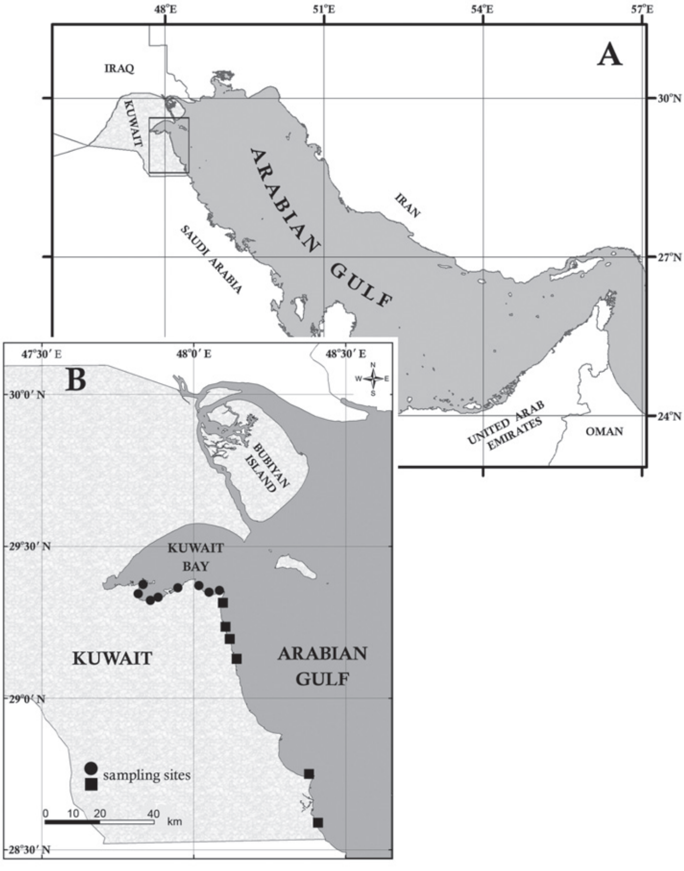

Figure 1. Area of investigation. **A** Arabian Gulf, with inset showing the greater region in which the sampling area was located **B** Map of Kuwait showing sampling sites (sites located within low-energy and high-energy zones are labeled with black dots and black squares, respectively).

software. All observations presented here are based on light microscope investigations of freshly-collected living cells.

We used the taxonomic classification scheme of Throndsen (1997), which was a partially modified classification of Christensen (1962, 1966). The recent taxonomic position of *Protaspis* is presented in accordance with Adl et al. (2005). Species names are listed in alphabetical order within each class (Table 1).

Results and discussion

Flagellated inhabitants of Kuwait's intertidal sediments were very diverse and mostly composed of sand-dwelling dinoflagellates, euglenids and cryptomonads. We identified a total of 67 flagellate species (Table 1, Figs 2–7); most of them being reported from Kuwait from the first time.

Table 1. List of species encountered during the survey of free-living flagellates associated with intertidal sediments along the coastline of Kuwait, 2005–2007. Abbreviations for substrate are as follows: 'M' – mud, 'MS' – muddy sand, 'S' – sand, 'P' – small saline ponds, 'MA' – macroalgae; categories of occurrence are: 'C' – common, 'F' – frequent, 'R' – rare; light microphotographs: figure numbers are followed by the numbers of the photographs.

Taxon	Substrate	Occurrence	Photograph
Class **Dinophyceae**			
Adenoides eludens (Herdman) Balech 1956	S	C	2:1–3
Amphidiniella sp.	S	R	2:4, 5
Amphidiniopsis arenaria Hoppenrath 2000	S	C	2:12, 13
Amphidiniopsis dentata Hoppenrath 2000	S	C	2:6–8
Amphidiniopsis swedmarkii (Balech) Dodge 1982	MS, S	C	2:9–11
Amphidinium carterae Hulbert 1957	MS, S	C	3:1
Amphidinium corpulentum Kofoid *et* Swezy 1921	S	R	3:19, 20
Amphidinium corrugatum Larsen *et* Patterson 1990	S	R	3:6–8
Amphidinium gibbosum (Maranda *et* Shimizu) Flø Jørgensen *et* Murray 2004	S	R	3:4
Amphidinium glabrum Hoppenrath *et* Okolodkov 2000	S	R	3:9, 10
Amphidinium herdmanii Kofoid *et* Swezy 1921	S	C	3:21, 22
Amphidinium incoloratum Campbell 1973	S	C	3:5
Amphidinium mootonorum Murray *et* Patterson 2002	S	C	3:15, 16
Amphidinium operculatum Claparède *et* Lachmann 1859	S	C	3:2
Amphidinium poecilochroum Larsen 1985	S	R	4:1
Amphidinium psittacus Larsen 1985	MS, S	F	4:2
Amphidinium scissum Kofoid *et* Swezy 1921	MS, S	C	3:11–14
Amphidinium semilunatum Herdman 1924	MS, S	C	3:18
Amphidinium steinii Lemmermann 1910	S	C	3:3
Amphidinium testudo Herdman 1924	S	R	3:17
Amphidinium sp. 1	S	R	4:3–5
Amphidinium sp. 2	S	R	4:6, 7
Bysmatrum teres Murray, Hoppenrath, Larsen *et* Patterson 2006	S	R	4:14–16
Coolia cf. *areolata* Ten-Hage, Turquet, Quod *et* Coutè 2000	S	R	2:16
Coolia monotis Meunier 1919	MA, S	R	2:14, 15
Gymnodinium venator Flø Jørgensen *et* Murray 2004	S	C	4:10, 11
Gyrodinium estuariale Hulburt 1957	M-S	C	4:8
Gyrodinium sp.	S	R	4:9
Herdmania litoralis (Dodge) Hoppenrath 2000	S	C	4:18, 19
Heterocapsa cf. *psammophila* Tamura, Iwataki *et* Horiguchi 2005	S	C	5:6–8
Heterocapsa sp.	S	C	4:12, 13
Katodinium asymmetricum (Massart) Loeblich 1965	MS, S	F	5:5
Katodinium glandula (Herdman) Loeblich 1965	S	C	5:1–4
Oxyrrhis marina Dujardin 1841	M-S	C	4:20, 21
Peridinium quinquecorne Abè 1927	S	R	4:17
Prorocentrum concavum Fukuyo 1981	MA, S	R	5:9–11
Prorocentrum fukuyoi Murray *et* Nagahama 2007	S	C	5:18, 19
Prorocentrum lima (Ehrenberg) Dodge 1975	MA, S	F	5:12, 13
Prorocentrum rhathymum Loeblich III, Sherley *et* Schmidt 1979	MA, S	R	5:14–17

Taxon	Substrate	Occurrence	Photograph
Roscoffia minor Horiguchi *et* Kubo 1997	S	R	5:20, 21
Sinophysis ebriolum (Herdman) Balech 1956	S	F	2:19
Sinophysis stenosoma Hoppenrath 2000	S	F	2:20
Thecadinium ovatum Yoshimatsu, Toriumi *et* Dodge 2004	S	R	2:17, 18
Class **Cryptophyceae**			
Platychilomonas psammobia Larsen *et* Patterson 1990	S	F	6:1, 2
Rhodomonas salina (Wislouch) Hill *et* Wetherbee 1989	MS, S	C	6:3–5
Class **Chlorophyceae**			
Dunaliella salina (Dunal) Teodoresco 1905	P	C	6:6, 7
Class Prasinophyceae			
Pyramimonas cf. *octopus* Moestrup *et* Kristiansen 1987	S	F	6:8, 9
Class Euglenophyceae			
Anisonema acinus Dujardin 1841	MS, S	C	6:12, 13
Chasmostoma nieuportense Massart 1920	M, MS	R	7:3
Dinema litorale Skuja 1939	S	F	6:10, 11
Dinema validum Larsen *et* Patterson 1990	M, MS	F	6:14–16
Eutreptia pertyi Pringsheim 1953	S	F	7:1
Eutreptiella sp.	S	R	7:2
Heteronema exaratum Larsen *et* Patterson 1990	MS	R	6:17, 18
Heteronema larseni Lee *et* Patterson 2000	S	C	6:19
Heteronema ovale Kahl 1928	S	F	6:20
Notosolenus ostium Larsen *et* Patterson 1990	MS, S	C	7:9
Petalomonas minor Larsen *et* Patterson 1990	M, MS	F	7:10, 11
Ploeotia heracleum Larsen *et* Patterson 1990	S	R	7:4
Ploeotia cf. *oblonga* Larsen *et* Patterson 1990	S	F	7:5
Ploeotia pseudoanisonema Larsen *et* Patterson 1990	M, MS	F	7:6, 7
Ploeotia sp.	S	F	7:8
Urceolus sabulosus Stokes 1886	M, MS	F	7:12–16
Cercozoa			
Protaspis grandis Hoppenrath *et* Leander 2006	S	C	7:17
Protaspis maior Skuja 1939	S	R	7:18
Protaspis obliqua Larsen *et* Patterson 1990	MS, S	C	7:19
Protaspis sp.	S	F	7:20, 21

The diversity of the flagellated group was mainly due to sand-dwelling dinoflagellates and euglenids. *Amphidinium*, with 17 species, was among the most abundant and diverse sand-dwelling dinoflagellate genera. Within this genus, the large, unarmored *A. scissum* was widely distributed throughout the year and may be the most abundant on Kuwait's intertidal sand flats. This species was occasionally found in other habitats.

A limited number of benthic dinoflagellates are potentially harmful, as they are capable of producing toxins, which may result in an intoxication of the marine environment. Among taxa of sand-dwelling dinoflagellates recorded in Kuwait were eight species that must be considered potentially harmful. *Prorocentrum concavum*

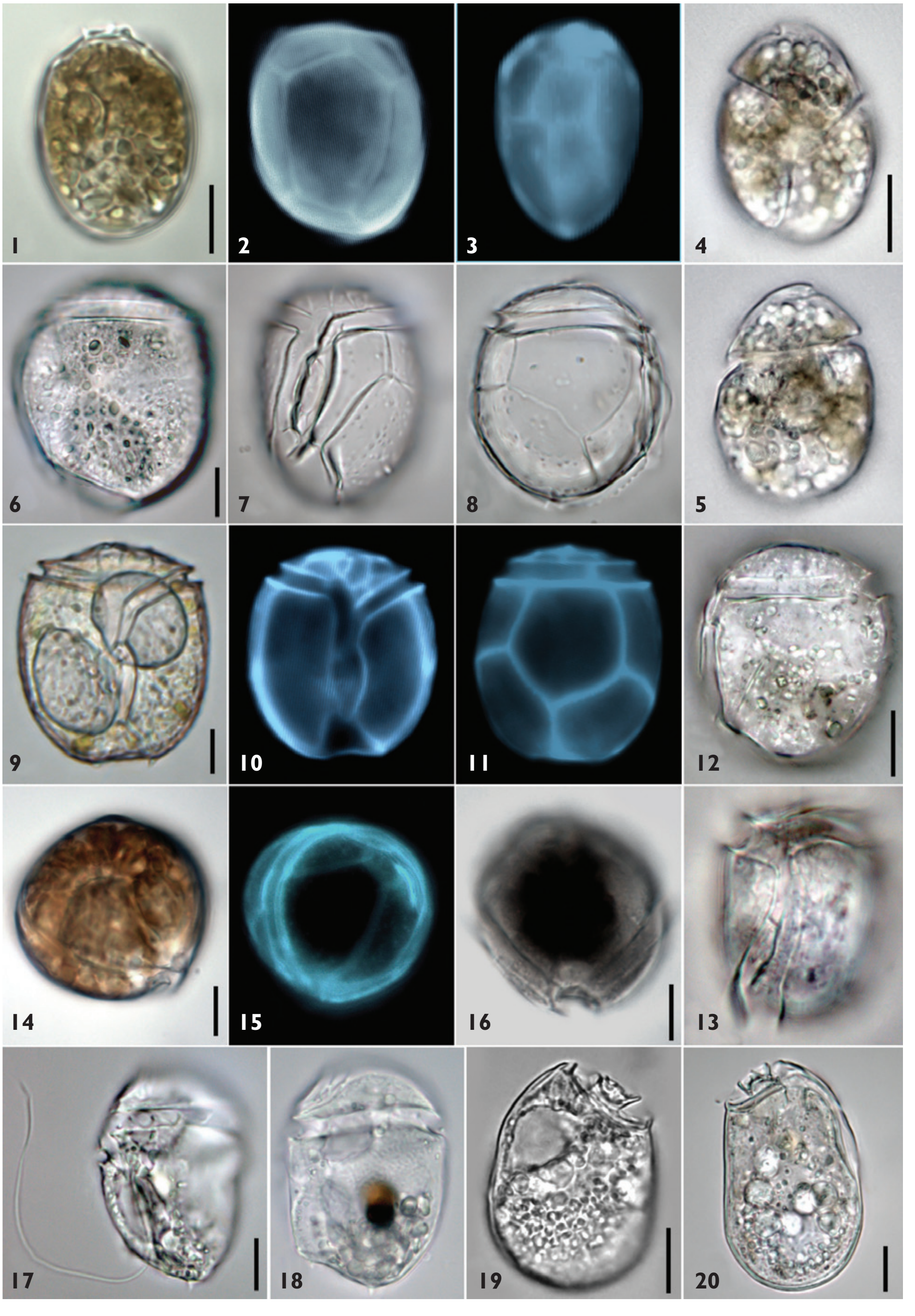

Figure 2. Light micrographs of the genera *Adenoides*, *Amphidiniella*, *Amphidiniopsis*, *Coolia*, *Thecadinium* and *Sinophysis*. **1–3** *Adenoides eludens* **4, 5** *Amphidiniella* sp. **6–8** *Amphidiniopsis dentata* **9–11** *Amphidiniopsis swedmarkii* **12, 13** *Amphidiniopsis arenaria* **14, 15** *Coolia monotis* **16** *Coolia* cf. *areolata* **17, 18** *Thecadinium ovatum* **19** *Sinophysis ebriolum* **20** *Sinophysis stenosoma*. Photos 2, 3, 10, 11, 15: epifluorescence. Scale bar = 10 µm for all photos.

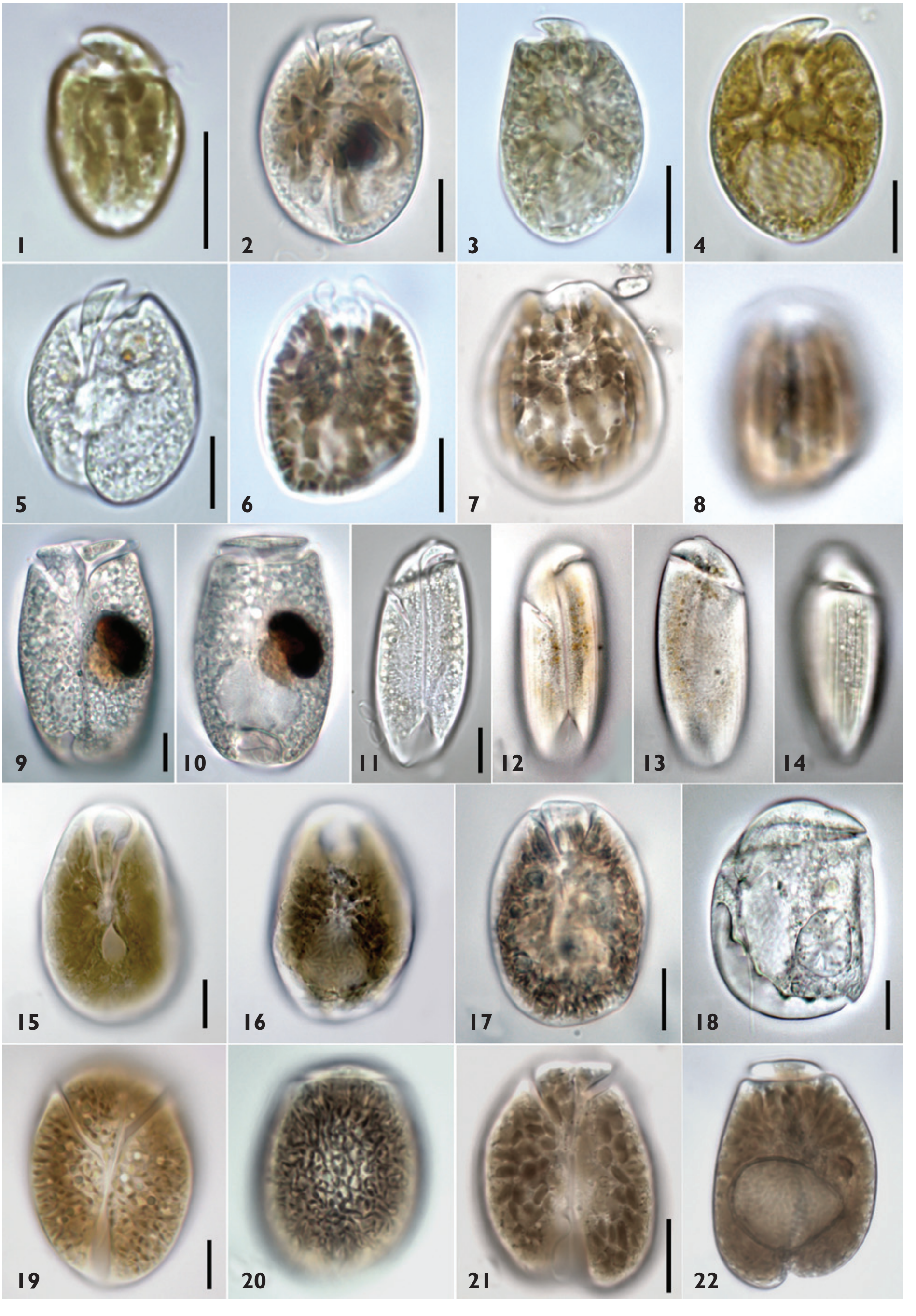

Figure 3. Light micrographs of the genus *Amphidinium*. **1** *Amphidinium carterae* **2** *Amphidinium operculatum* **3** *Amphidinium steinii* **4** *Amphidinium gibbosum* **5** *Amphidinium incoloratum* **6–8** *Amphidinium corrugatum* **9, 10** *Amphidinium glabrum* **11–14** *Amphidinium scissum* **15, 16** *Amphidinium mootonorum* **17** *Amphidinium testudo* **18** *Amphidinium semilunatum* **19, 20** *Amphidinium corpulentum* **21, 22** *Amphidinium herdmanii*. Scale bar = 10 µm for all photos.

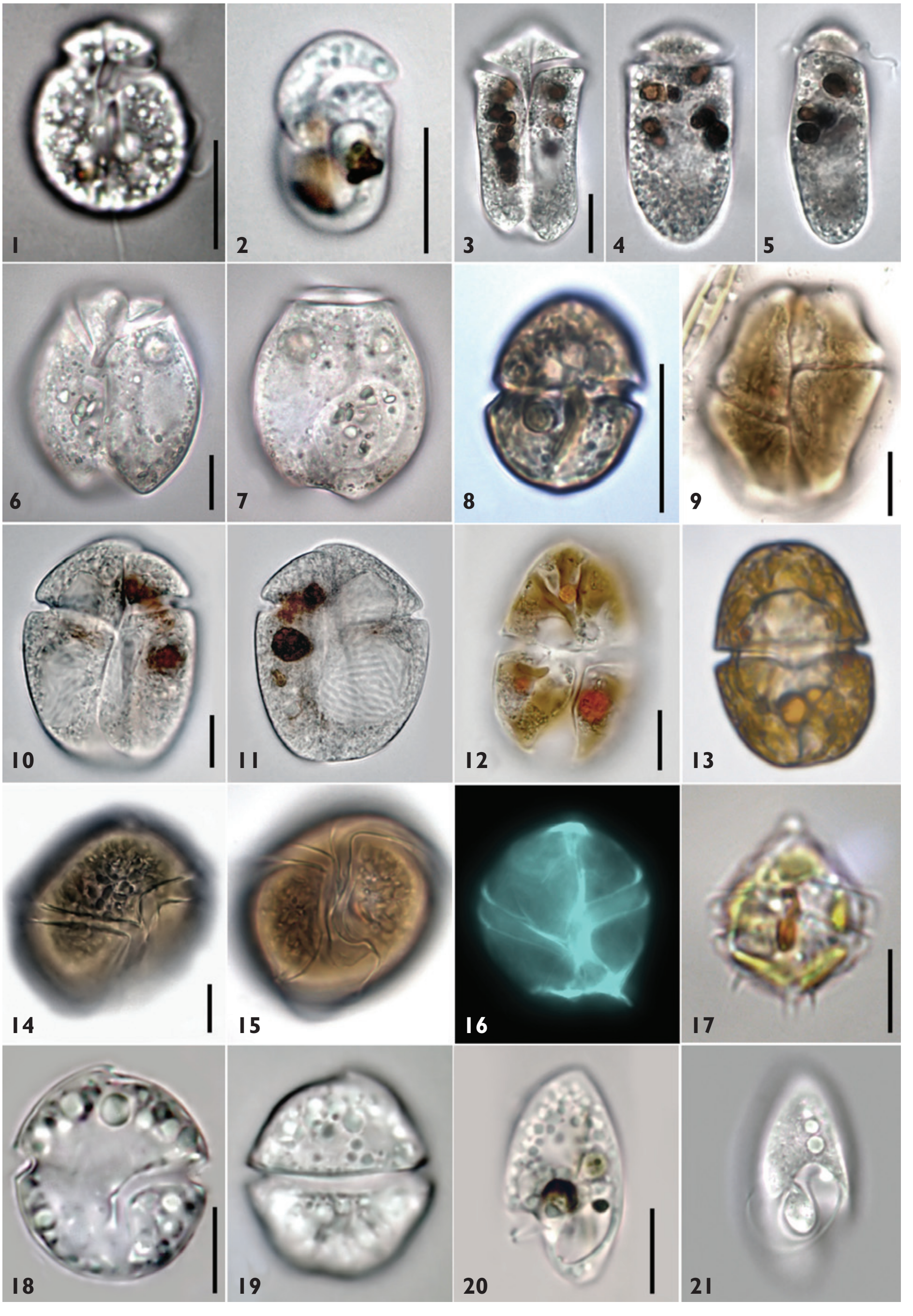

Figure 4. Light micrographs of the genera *Amphidinium*, *Gyrodinium*, *Gymnodinium*, *Heterocapsa*, *Bysmatrum*, *Peridinium*, *Herdmania* and *Oxyrrhis*. **1** *Amphidinium poecilochroum* **2** *Amphidinium psittacus* **3–5** *Amphidinium* sp. 1 **6, 7** *Amphidinium* sp. 2 **8** *Gyrodinium estuariale* **9** *Gyrodinium* sp. **10, 11** *Gymnodinium venator* **12, 13** *Heterocapsa* sp. **14–16** *Bysmatrum teres* **17** *Peridinium quinquecorne* **18, 19** *Herdmania litoralis* **20, 21** *Oxyrrhis marina.* Photo 16: epifluorescence. Scale bar = 10 µm for all photos.

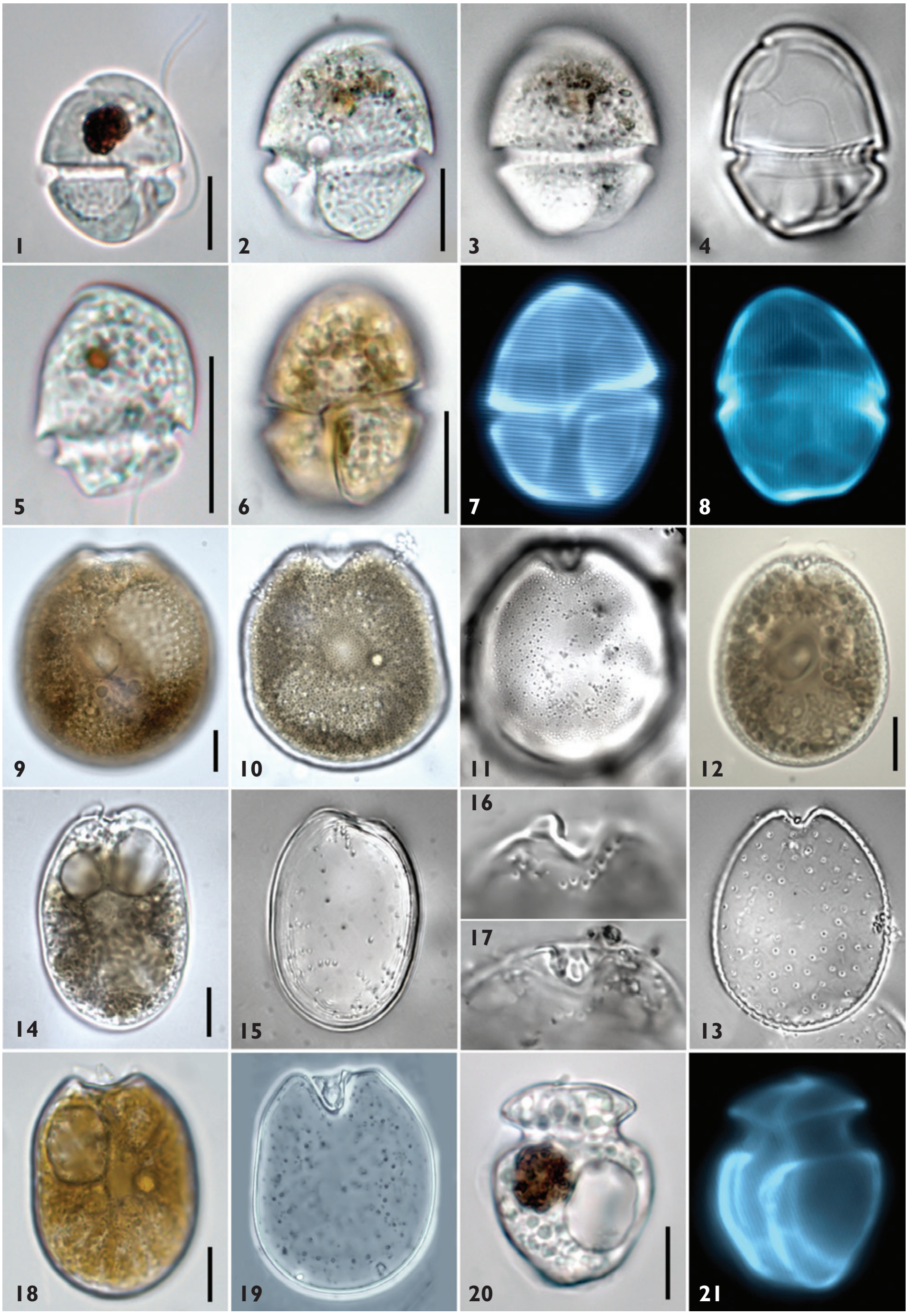

Figure 5. Light micrographs of the genera *Katodinium*, *Heterocapsa*, *Prorocentrum* and *Roscoffia*. **1–4** *Katodinium glandula* **5** *Katodinium asymmetricum* **6–8** *Heterocapsa* cf. *psammophila* **9–11** *Prorocentrum concavum* **12, 13** *Prorocentrum lima* **14–17** *Prorocentrum rhathymum* **18, 19** *Prorocentrum fukuyoi* **20, 21** *Roscoffia minor.* Photos 7, 8, 21: epifluorescence. Scale bar = 10 µm for all photos.

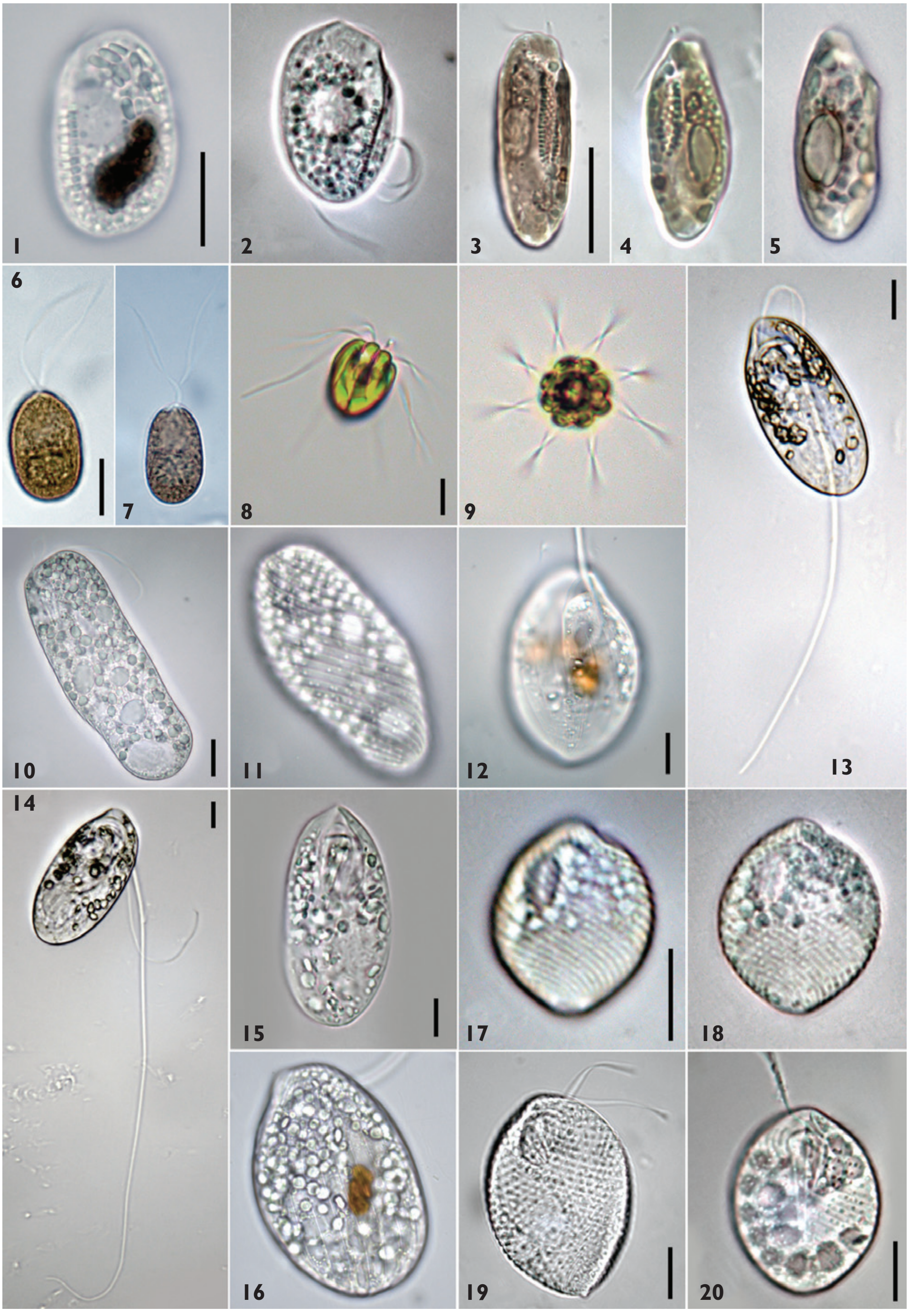

Figure 6. Light micrographs of the genera *Platychilomonas*, *Rhodomonas*, *Dunaliella*, *Pyramimonas*, *Dinema*, *Anisonema* and *Heteronema*. **1, 2** *Platychilomonas psammobia* **3–5** *Rhodomonas salina* **6, 7** *Dunaliella salina* **8, 9** *Pyramimonas* cf. *octopus* **10, 11** *Dinema litorale* **12, 13** *Anisonema acinus* **14–16** *Dinema validum* **17, 18** *Heteronema exaratum* **19** *Heteronema larseni* **20** *Heteronema ovale.* Scale bar = 10 μm for all photos.

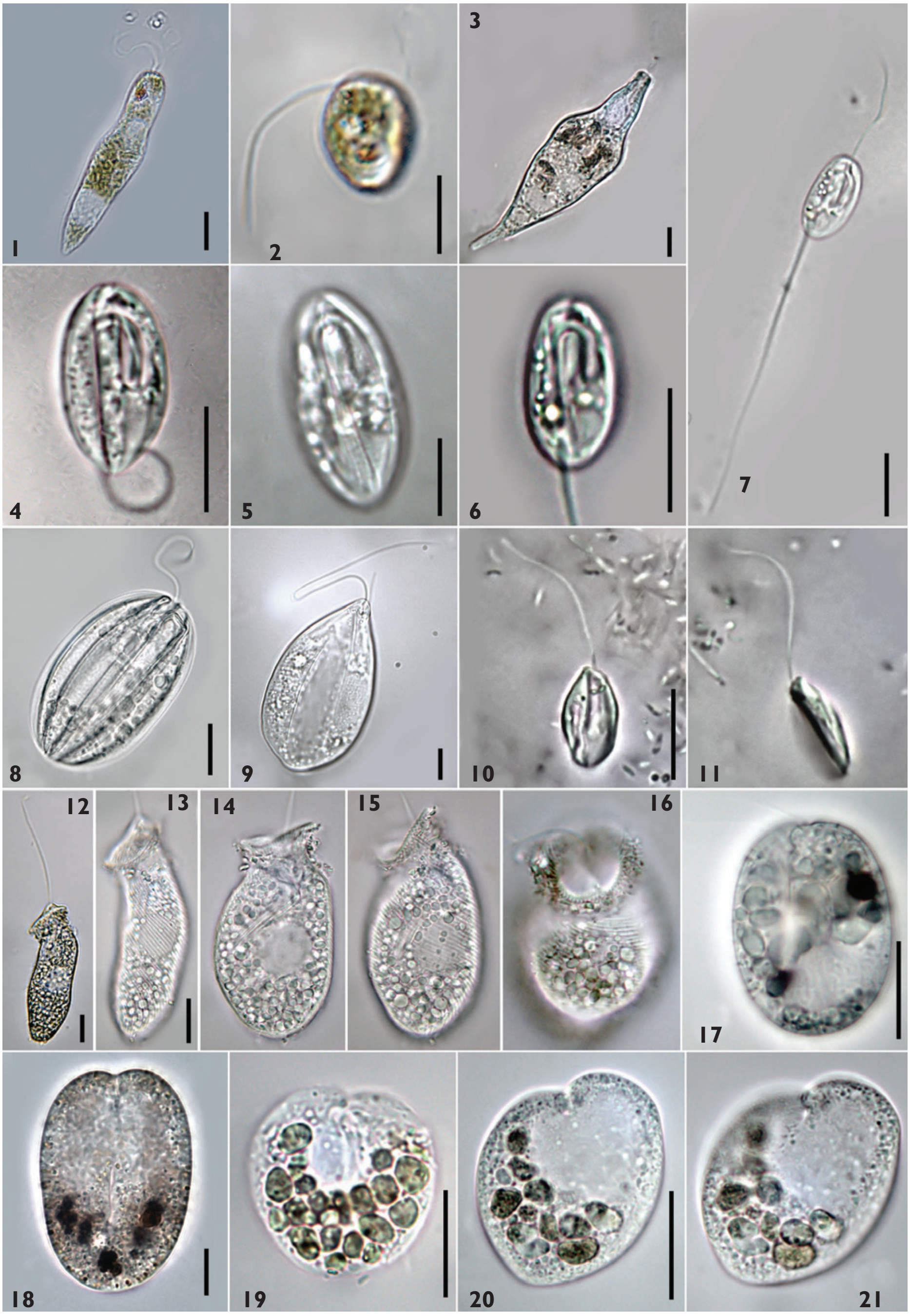

Figure 7. Light micrographs of the genera *Eutreptia*, *Eutreptiella*, *Chasmostoma*, *Ploeotia*, *Notosolenus*, *Petalomonas*, *Urceolus* and *Protaspis*. **1** *Eutreptia pertyi* **2** *Eutreptiella* sp. **3** *Chasmostoma nieuportense* **4** *Ploeotia heracleum* **5** *Ploeotia* cf. *oblonga* **6, 7** *Ploeotia pseudoanisonema* **8** *Ploeotia* sp. **9** *Notosolenus ostium* **10, 11** *Petalomonas minor* **12–16** *Urceolus sabulosus* **17** *Protaspis grandis* **18** *Protaspis maior* **19** *Protaspis obliqua* **20, 21** *Protaspis* sp. Scale bar = 10 μm for all photos.

has been reported to produce three diol esters of okadaic acid and ichthyotoxin (Yasumoto et al. 1987, Hu et al. 1993). *Prorocentrum lima* may produce okaidic acid and dinophysistoxins, which may cause diarrhetic shellfish poisoning (Murakami et al. 1982, Tindall et al. 1984, Torigoe et al. 1988, Lee et al. 1989, Hu et al. 1993). *Prorocentrum rhathymum* may produce water-soluble fast-acting toxins and hemolytic effects (Nakajima et al. 1981, Tindall et al. 1989). In the summer of 1999, a bloom of *P. rhathymum* (reported as *P. mexicanum*) caused a massive fish kill in Kuwait Bay (Al-Yamani et al. 2004). *Coolia monotis* is known to produce cooliatoxin, which is presumably related to yessotoxin (Holmes et al. 1995). It may be involved in ciguatera (Tindall and Morton 1998). Haemolysins, compounds toxic to fish, have been isolated from *Amphidinium carterae* (Yasumoto et al. 1987). *Amphidinium gibbosum* may produce cytotoxic metabolites, the most potent of which (caribenolide I) had anti-tumor effects (Bauer et al. 1994, Bauer et al. 1995a, b, Maranda and Shimizu 1996). Haemolytic and antifungal properties (amphidinols) are reported from *A. operculatum*; it may also be toxic to fish (Yasumoto et al. 1987). *Peridinium quinquecorne* can cause anoxia and fish kills, if occurring with very high cell densities (Fukuyo et al. 1990). These potentially harmful dinoflagellates were present in Kuwait's benthic microalgal community during 2005 to 2007, but never occurred in great numbers.

Euglenids comprised auto- and heterotrophic species. Among them, *Anisonema acinus* was the most common and widespread species in Kuwait's muddy and sandy sediments. *Petalomonas minor* and *Urceolus sabulosus* were mainly recorded in intertidal mudflats of Kuwait Bay, where they can be quite abundant.

The highest species diversity of flagellates in Kuwait's soft sediments was associated with the southern intertidal sand flats. Together with benthic diatoms and cyanobacteria, autotrophic flagellates are likely to be among the most important contributors to primary productivity in the intertidal zone. Heterotrophic species may play an important role in intertidal food webs, consuming even large diatoms and other flagellates.

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